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Research Article

Influence Of Anionic, Cationic And Non-Ionic Surfactants On Growth Of Hydrocarbon Utilizing Bacteria

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Abstract

The effects of named anionic, cationic and non-ionic surfactants on growth of hydrocarbon utilizing bacteria were studied. Hydrocarbon utilizing bacteria were isolated from soil samples collected from different sampling points using mineral salt medium supplemented with hydrocarbon substrates. They were identified based on 16S rDNA sequencing. Hydrocarbons used include crude oil, kerosene, diesel and spent-engine oil. Heterotrophic bacterial count was taken to assess the bacterial count and hydrocarbon utilization of the tested hydrocarbons. Effects of Sodium dodecyl sulphate (anionic surfactant), Cetyl trimethyl ammonium bromide(cationic surfactant) and Tween-80 (non-ionic surfactant) surfactants at 0.05 - 0.30 mg/ml concentrations, on growth of the isolates were measured spectrophotometrically. Results obtained showed high bacteria count of $12x10^3$Cfu/ml with spent oil and least (2×10^3Cfu/ml) with diesel. Five bacteria belonging to the genera *Micrococcus, Serratia, Pseudomonas, Staphylococcus and Bacillus* were isolated. Tween-80 stimulated the growth of all the isolates. While SDS stimulated growth of *Micrococcus luteus, Staphylococcus scuiri and Bacillus cereus*, it reduced the growth of *Serratia marcescens* and *Pseudomonas aeruginosa.* CTAB was observed to improve the growth of only *Pseudomonas aeruginosa*. and inhibited the growth of other isolates. Statistical analysis indicates that there was significant difference on the effects of the surfactants on the growth of the isolates with p-value < 0.0001. Surface active agents increases or inhibits microbial growth at certain concentration. The use of surfactant in biostimulation of hydrocarbon degrading microorganisms is recommended. Study on the correct concentration required for growth of the microorganisms should be conducted for its efficiency and effectiveness.

Keywords: Anionic surfactant; cationic surfactant; non-ionic surfactant; microbial growth; hydrocarbon utilization
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Introduction

The term surfactant is a blend of "surface active agents". Surfactants are usually organic compounds that are amphiphilic, that is, they contain both hydrophobic tails and hydrophilic heads. The hydrophobic and hydrophilic moieties partition at the interface between fluid phases with different degrees of polarity and hydrogen bonding such as oil/water or air/water interfaces [1]. These properties render surfactants capable of reducing surface and interfacial tension and forming microemulsion where hydrocarbons can solubilize in water or where water can solubilize in hydrocarbons. Such characteristics confer excellent detergency, emulsifying, foaming, and dispersing traits, which make surfactants one of the most versatile process chemicals [2]. Chemically synthesized surfactants are commonly used in petroleum, food and pharmaceutical industries as emulsifiers, wetting agents, and foaming agents.

Surfactants used for bioremediation is utilized by the microbial population as growth substrate resulting in increased microbial biomass and enhanced contaminant removal. The use of surfactants was proposed to enhance bioremediation of hydrocarbon contaminated sites [3,4]. In some cases, the microbial population could adopt the surfactants used for bioremediation and transform them to growth substances which could lead to an increase in the level of the biomass [5].

Bacteria that can degrade petroleum products are species of *Pseudomonas, Aeromonas, Moraxella, Beijerinckia, Flavobacterium, Chromobacterium, Nocardia, Corynebacterium, Acinetobacter, Mycobacterium, Streptomyces, Bacillus, Arthrobacter,Aeromonas, Cyanobacterium.* Some microorganisms capable of degrading hydrocarbon substrates excrete biosurfactants which aids the microorganism by increasing the surface area and bioavailability of the water-insoluble substrates. Anaukwu et al. [6] isolated *Pseudomonas monteilii* and *Citrobacter murliniae* capable of producing biosurfactant. *Bacillus subtilis, Pseudomonas aeruginosa, Torulopsis bombicola* have also been reported to utilize crude oil and hydrocarbons by producing biosurfactant [7].

The objectives of our study were to isolate and characterize hydrocarbon utilizing bacteria, and to study the influence of varying concentrations of different classes of surfactants on the growth of the isolated hydrocarbon utilizers.

Materials and methods

Sample collection

Soil samples were randomly collected from the rhizosphere of plants, mechanic workshops, petrol stations and refuse dump sites at the soil depth of 2-8cm in Awka, Anambra state, Nigeria, with a sterilized screw capped bottles. They were analyzed within 24hr of sampling in the microbiology laboratory.
Hydrocarbons used for this study are crude oil, kerosene, diesel and spent engine oil.

Isolation and Screening of Hydrocarbon Utilizers

The method described by Ezemba et al.[8] was used for enrichment of hydrocarbon-degrading bacteria. Two grams of soil sample was serially diluted in ten-folds in sterile distilled water. 1% hydrocarbon and 0.1ml of 10^{-3} dilutions of the sample were introduced into 10ml of sterile mineral salt medium consisting of: NaCl ,0.4g; NH_4Cl ,0.5g; $MgSO_4.7H_2O$,0.5g; KH_2PO_4 ,0.05g, distilled H_2O ,I L and $NaHPO_4.7H_2O$,0.05g. The tube was incubated in a rotary shaker at 30^oC for 7 days.

Hydrocarbon-utilizing bacteria were isolated from the enrichment culture by inoculating 1ml of the culture onto mineral salt medium with 2% agar by pour plate technique. 0.5ml of hydrocarbon was soaked into a sterile filter paper and aseptically placed on the lids of 70 by 15mm-diameter sterile Petri dishes. The dishes containing the medium inoculated with the isolates were then inverted and incubated at 30^{o}C for 5 days. Heterotrophic bacterial count was taken after incubation and pure isolates were transferred onto Nutrient agar (Oxoid) slants and stored at 4^{o}C for further characterization.

Characterization of Isolate

Hydrocarbon utilizing isolates were identified based on 16S rDNA sequencing using the FASTA algorithm with the Prokaryote database from European Bioinformatics.

Effect of surfactant on growth of isolates

Surfactants investigated were SDS (anionic surfactant), cetyl trimethyl ammonium bromide [CTAB] (cationic surfactant) and tween-80 (non-ionic surfactant). The effect of different concentrations (0.05-0.3mg/ml) of SDS, CTAB and tween-80 on the growth of the isolates was studied. 1ml of 24h broth culture of each of the isolates were introduced into sterile test tube containing 9ml of the mineral salt medium, 1% hydrocarbon and varying concentration of each surfactant. The test tubes were incubated at 30^{o}C for 48hrs. Growth of the isolates were read spectrophotometrically at 600nm. Triplicate tubes were used. Uninoculated test tube served as control.

Results

The heterotrophic bacterial count of hydrocarbon utilizing bacteria grown in mineral salt medium supplemented with various hydrocarbon is shown in Fig.1. The count ranges from 2 × 10^{3} Cfu/ml to 12 × 10^{3}Cfu/ml. The highest count was observed with spent engine oil and least with diesel. The hydrocarbon utilizers isolated in this study belong to the genera *Micrococcus, Serratia, Pseudomonas, Staphylococcus and Bacillus.*

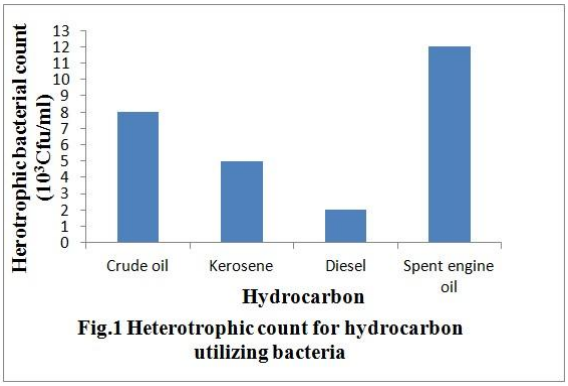

Fig.1 Heterotrophic count for hydrocarbon utilizing bacteria

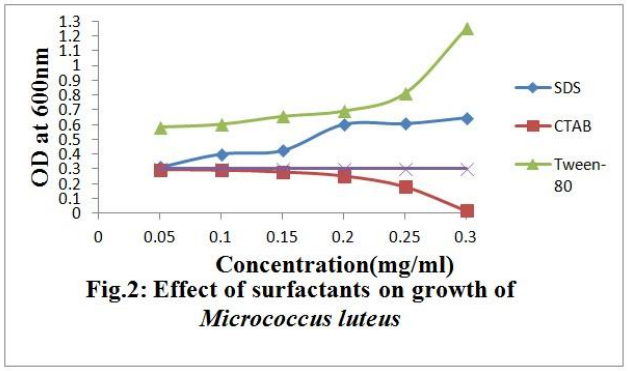

Fig.2: Effect of surfactants on growth of *Micrococcus luteus*

Fig. 2 - 6, show the effect of Surfactants on the isolates. Tween-80 enhanced the growth of the isolates at all concentrations. While SDS stimulated growth of *Micrococcus luteus, Staphylococcus scuiri and Bacillus cereus*, it reduced the growth of *Serratia marcescens* and *Pseudomonas aeruginosa.* CTAB was observed to

improve the growth of only *Pseudomonas aeruginosa* while growth of other isolates were inhibited at all concentrations.

There was gradual increase in growth of *Micrococcus luteus* with increasing concentration of Tween-80 (Fig.2), with highest growth of 1.251 at concentration of 0.3mg/ml. CTAB gave an inhibitory effect on the growth of *M. luteus* with increasing concentration. There was significant difference in the effects of Tween-80 and SDS on growth of *M. luteus* with p-value < 0.0001.

Serratia marcescens was greatly inhibited at all concentrations of SDS and CTAB (Fig.3). At concentrations of 0.1mg/ml and 0.2mg/ml, there were no significant difference in their effects on the growth of *S. marcescens* with p-value = 0.245.

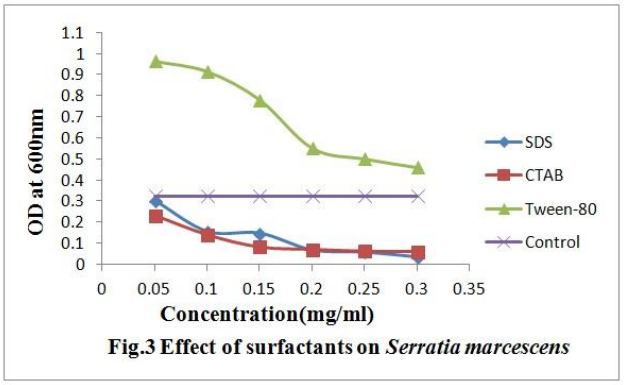

Fig.3 Effect of surfactants on *Serratia marcescens*

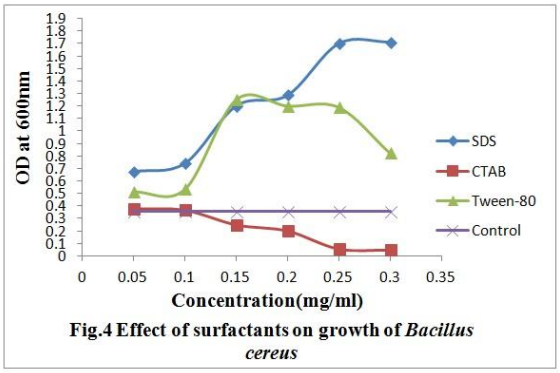

Fig.4 Effect of surfactants on growth of *Bacillus cereus*

Fig.4 shows a logarithmic increase in growth of *Bacillus cereus* with increasing concentration of SDS and Tween-80. *Pseudomonas aeruginosa* was greatly stimulated in the presence of CTAB with increasing concentration of the surfactant (Fig.5).

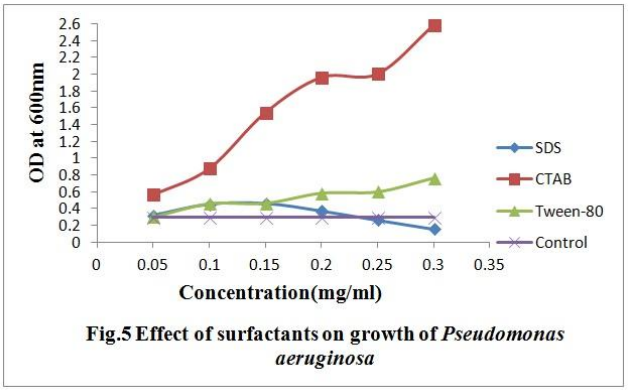

Fig.5 Effect of surfactants on growth of *Pseudomonas aeruginosa*

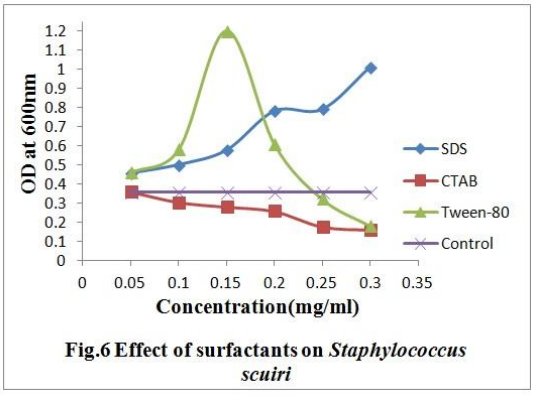

Fig.6 Effect of surfactants on *Staphylococcus scuiri*

Fig.6 shows that Tween-80 stimulated growth of *Staphylococcus scuiri* at low concentrations, increased concentration above 0.15mg/ml resulted in decrease in the bacterial growth. Statistical analysis indicates that there was significant difference on the effects of the surfactants on the growth of the isolates with p-value <0.0001.

Discussion

Hydrocarbon utilizing bacteria have the ability of degrading hydrocarbon. The isolation of *Micrococcus luteus*, *Serratia marcescens*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus scuiri* as hydrocarbon utilizers in this work is in line with the work of Okareh et al.[5], who isolated *Flavobacterium* and *Corynebacterium* alongside *Pseudomonas, Bacillus* and *Micrococcus* in their work on hydrocarbon-degrading bacteria isolation and surfactant influence on the growth of organisms. Mbachu et al.[9], in their work also isolated *Pseudomonas* sp., and *Bacillus* sp. alongside *Acinetobacter, Corynebacterium* sp. and *Flavobacterium* sp. as hydrocarbon degrading bacteria.

Surfactants are well known surface active agents that are generally used to increase the surface area for microbial action and availability of nutrients to the microorganisms [10]. Several kinds of surfactants are known to affect permeability in microorganisms [11,12].

The stimulatory effects of the Tween-80 reported in this work is supported by the works of Jing-Yan et al.[13] and Van Boxel et al.[14]. While Jing-Yan et al.[13], reported that Tween 80 showed significant effect on growth and production of *cis*-9, *trans*-11 conjugated linoleic acid production of *Lactobacillus acidophilus* F0221, Van Boxel et al.[14], reported that Tween-80 enhanced the growth of *Mycobacterium paratuberculosis* at concentration range of 0-0.01% and 0.1-1.0% (w/v). The growth enhancement of Tween-80 was probably due to its ability to increase solubility of the bacterial surface component thus altering cell wall permeability and facilitating transport of substrates into the cell.

Sodium dodecyl sulfate (SDS) has been described as an effective anionic surfactant for removing hydrophobic contaminants from contaminated sand [15]. Its ability to improve growth of Gram positive hydrocarbon utilizers in this study can be seen in its ability to enhance bioavailability of hydrophobic compounds present in the medium. Margesin and Schinner [16], reported that SDS at low concentrations (50 -100 mg /1) significantly enhanced growth and oil biodegradation by a psychrotrophic inoculum in liquid culture.

CTAB enhanced the growth of *Pseudomonas aeruginosa* but inhibited the growth of other isolates. Cetyl trimethyl ammonium bromide (cetrimide) is known for inhibiting growth of microorganisms but can be metabolized by *Pseudomonas* species, thus the use of cetrimide-containing medium as a selective medium for *Pseudomonas* species. This is supported by the work of Yalcin et al.[17], who noted increase in growth profile of *Pseudomonas putida* RW-11 by 14.4% in the presence of CTAB.

The use of surfactant in biostimulation of hydrocarbon degrading microorganisms is recommended.

Conclusion

The results obtained in this study revealed that non-ionic surfactant(Tween-80) stimulated growth of all the isolates. While anionic surfactant (SDS) stimulated growth of some of the isolates and inhibited others at some concentrations, cationic surfactant (CTAB) inhibited growth of all the isolates except *pseudomonas aeruginosa.* However, study on the correct concentration of these surfactants is required for their effectiveness.

Conflict of Interest

The authors declare that they have no conflict of interest.

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